Comparison of Estimates of Ruminal Protein Degradation by In Vitro and In Situ Methods

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COMPARISON OF ESTIMATES OF RUMINAL PROTEIN DEGRADATION BY IN VITRO AND IN SITU METHODS[1,2]

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ABSTRACT

Ruminal degradation of eight soluble proteins and 10 protein meals was determined using three methods: 1) an inhibitor in vitro system (IIV), to which inhibitors are added to prevent metabolism of protein degradation products, 2) in situ incubations in nylon bags and 3) in vitro NH_3 production in typical ruminal inoculum. In vitro NH_3 production rate from different proteins was not related to in situ or IIV degradation rate. Degradation rates for soluble proteins by IIV ranged from .103 to .813/h, yielding estimated extents of degradation that ranged from 73 to 94% (assuming ruminal passage of .05/h). For seven of the protein meals, degradation rates measured by IIV were threefold greater than in situ rates. However, mean degraded fractions estimated from zero-time intercepts were twofold greater using the in situ method, and calculated extents of degradation averaged 83% of IIV values. Extents of degradation estimated (assuming ruminal passage of .05/h) for fish meal, soybean meal, linseed meal, sunflower meal, rapeseed meal, copra meal and meat and bone meal were, respectively; 55, 79, 84, 59, 75, 54 and 58% (IIV) and 46, 63, 69, 51, 52, 43 and 55% (in situ). These values were generally similar to those reported in the literature. The extent of degradation of feather meal was 28% by in situ determination. Neither the IIV nor in situ method gave significant regressions for blood meal; neither method yields reliable data for very slowly degraded proteins. The IIV procedure has the advantage over the in situ method because it can yield degradation estimates for soluble as well as insoluble proteins.
(Key Words: Rumen, Degradation, Solubility, Protein Sources.)

Introduction

Dietary proteins vary greatly in ruminal degradation. Proteins with low degradation are especially valuable to ruminants with high protein requirements as dairy cows in early lactation and early-weaned calves and lambs. Other ruminants that have been reported to respond to additional nondegraded protein are wool-producing sheep fed at maintenance and growing cattle fed high non-protein N diets containing little preformed protein. The amino acid pattern or total protein supply frequently is inadequate to meet requirements of these animals.

New feeding systems place emphasis on quantifying ruminal protein degradation (ARC, 1984; NRC, 1985). Therefore, it is necessary to assess rapidly and accurately the degradation of feed proteins. The procedure gaining widest application is the in situ bag technique: the protein under study is contained in synthetic fiber bags suspended within the rumen, and the rate of N loss from the bags is used to determine rate and extent of protein degradation (Mehrez and Ørskov, 1977). Although used extensively, in situ techniques have been criticized (Meyer and Mackie, 1986; Nocek and Grant, 1987). One of the difficulties of some in vitro systems has been incomplete recovery of degradation products (Broderick, 1982; Miller, 1982). Recently, a revised in vitro method was

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reported that incorporated inhibitors of microbial N metabolism, thus allowing quantitative recovery of protein degradation products (Broderick, 1987). The purpose of our research was to compare this new method with a conventional in situ procedure for estimating rates and extents of ruminal protein degradation.

Materials and Methods

The proteins studied were from the following sources: casein was used as purchased[5] or after precipitation and freeze-drying (Blackburn and Hobson, 1960); pot ale syrup and spent wash, by-products of Scotch whisky production[6], were freeze-dried; four samples of fish solubles (fish solubles I to IV, each judged by the manufacturer to be of different quality) and one sample each of "nonruminant" and "ruminant" fish meal[7] were used as purchased. Soybean meal, linseed meal, sunflower meal, rapeseed meal, copra meal, meat and bone meal, feather meal and blood meal all were obtained commercially in Aberdeen, Scotland. Protein meals were ground through a 2.5-mm screen. All samples were analyzed (AOAC, 1980) for total Kjeldahl-N and dry matter (DM; 105°C); total amino acids were determined in acid hydrolysates (Broderick, 1987). The proteins also were assayed for water-soluble N by suspending in water for 30 min at room temperature and filtering through Whatman No. 1 paper. Low molecular weight N compounds were determined as the N that diffused through dialysis tubing with a 12,000 dalton cut-off.

The in situ protein degradation procedure was that outlined by Ganev et al. (1979). Five-gram air-dry samples were weighed into 100-mm × 170-mm nylon fiber bags (13 mg DM/cm^2) with a pore size of 50 × 80 (SD = 18) microns. Bags were suspended for periods of 4, 8, 16 and 24 h in the rumens of three sheep fed a diet based on ryegrass hay; zero-time N loss was not measured directly. Bags were washed and residual N determined by the method of Davidson et al. (1970). The in situ method was applied only to the meals that contained largely insoluble proteins. The exponential model of Ørskov and McDonald (1979) was used to compute the rates of protein degradation and extents of degradation assuming ruminal passage rates (k) of .02, .05 and .08/h.

Rates and extents of ruminal protein degradation were estimated by the inhibitor in vitro (IIV) method described by Broderick (1987) assuming ruminal passage rates (k_p) of .02, .05 and .08/h. In this procedure, the inhibitors hydrazine and chloramphenicol are incorporated into the medium to give quantitative recovery of NH_3 and amino acids produced during protein degradation. The fractions degraded and remaining undegraded for each protein are computed from release of NH_3 and total amino acids, based on the N and total amino acid content (Table 1) of that protein (Broderick, 1987). Incubations were conducted at 39°C for 4 h with sampling every .5 h; activity was stopped by the addition of trichloroacetic acid (TCA) and cetyltrimethylammonium bromide, dissolved in methylene chloride (Broderick, 1987). Protein was added at .125 mg N/ml medium; three separate incubations were conducted using mixed ruminal organisms at an average concentration of 8.64 mg microbial DM/ml. All 18 proteins were tested with this system.

Ammonia production in a conventional in vitro system also was determined for all proteins except commercial casein. Protein sources were added (20 mg DM/ml) to strained ruminal fluid obtained from a ruminally cannulated wether fed a mixed diet (Whitelaw et al., 1983). Incubations were conducted at 39°C and stopped after 4 h by addition of 5% w/v TCA; NH_3 concentrations were determined (Weatherburn, 1967). Net (i.e., blank-corrected) NH_3 production was expressed as mg NH_3N produced·h^{-1} g N added^{-1}.

Rates of degradation and intercepts for both in situ and IIV methods were computed using linear regression on time (Steel and Torrie, 1960) of the natural log of the fraction remaining undegraded. Regression also was used to determine correlations (Steel and Torrie, 1960). Computation of standard errors of IIV degradation rates and replication during in vitro incubations were as described previously (Broderick, 1987).

Results and Discussion

Analytical and in vitro degradation data obtained for all 18 proteins are given in Table 1. Ammonia production from proteins incubated with unmodified ruminal fluid in vitro

[5]Sigma Chemical Co., St. Louis, MO.
[6]Pentland Research, Ltd., Aberdeen, Scotland.
[7]Caledonian Fish Meal Co., Ltd., Aberdeen, Scotland.

TABLE 1. CHEMICAL ANALYSES, INHIBITOR IN VITRO (IIV) DEGRADATION DATA AND NH_3 PRODUCTION FROM 18 PROTEIN SOURCES

Protein	Total-N (DM[a] basis), %	TAA[a], μmol/mg N	WSN[b], %	Low MWN[c], %	IIV Degradation rate (k_d),h^{-1} (± SE)[a]	IIV *intercept* (B), %	IIV *degradability*[d], %	NH_3-N production[e], $Mg \cdot h^{-1} \cdot g\ N^{-1}$
Casein	15.53	46.5	100	ND	.489 ± .054	101.7	91	ND
Casein (freeze-dried)	13.43	48.2	100	8.0	.813 ± .110	98.4	94	11.0
Pot ale syrup	4.55	35.8	100	40.8	.103 ± .010	81.9	73	13.4
Spent wash	4.89	35.3	100	21.5	.145 ± .010	81.8	79	12.8
Fish solubles								
I	11.86	50.7	100	28.7	.594 ± .050	92.7	93	23.0
II	12.26	49.0	100	22.1	.550 ± .046	92.5	92	21.9
III	11.34	49.3	100	24.6	.584 ± .052	95.7	92	21.9
IV	11.19	50.0	100	24.9	.538 ± .048	93.1	92	22.2
Fish meal (nonruminant)	11.05	50.7	46.5	4.7	.078 ± .009	78.5	69	14.4
Fish meal (ruminant)	11.04	48.9	27.0	3.3	.042 ± .009	81.9	55	8.3
Soybean meal	7.62	43.8	43.7	10.0	.166 ± .019	92.7	79	7.5
Linseed meal	5.69	47.8	29.0	1.8	.244 ± .034	94.7	84	19.2
Sunflower meal	4.53	44.6	30.7	9.0	.058 ± .008	87.7	59	−10.1
Rapeseed meal	6.40	42.0	29.7	10.0	.124 ± .011	85.9	75	8.5
Copra meal	3.25	42.1	39.3	12.7	.050 ± .007	92.2	54	−7.5
Meat & bone meal	7.67	52.6	29.3	8.0	.056 ± .011	88.2	58	11.2
Feather meal[f]	13.27	53.6	25.3	4.2				2.2
Blood meal[f]	14.25	50.5	17.7	1.4				.9

[a]DM = Dry matter; TAA = Total amino acids; SE = standard error of the mean; ND = not determined.

[b]Water-soluble N, proportion of total N. Values of "100" were based on dry matter solubilities and were not determined directly.

[c]Low molecular weight N; proportion of total N in compounds of molecular weight less than 12,000 daltons.

[d]Extent of degradation of protein, % = $(100-B) + B[k_d/(k_d + k_p)]$, where $B \leq 100$ and the assumed ruminal passage rate, k_p = .05/h (Broderick, 1987).

[e]In vitro NH_3 production in ruminal fluid (without added inhibitors).

[f]Neither feather meal nor blood meal yielded significant IIV degradation regressions, so degradation parameters could not be computed.

did not have a strong correlation to degradation estimates made by the IIV method (r^2 = .352, Sy·x = 8.35, n = 15; Table 1) or by the in situ procedure (r^2 = .229, Sy·x = 9.22, n = 8; Table 2). Ammonia released in unmodified ruminal fluid can be assimilated into microbial protein, and the extent to which this occurs will depend on the fermentability of the feed. The inhibitors hydrazine and chloramphenicol prevent this incorporation in the IIV system.

Degradation rates for the eight soluble proteins with the IIV method were very rapid, typically about .5/h or faster. Exceptions were the proteins in pot ale syrup and spent wash (Table 1), which were degraded at .103 and .145/h, respectively. Both are by-products from Scotch whisky distilleries, and these slower degradations may reflect largely the degradation of soluble proteins from barley and yeast sources. It was anticipated that soluble proteins containing high levels of low molecular weght N compounds would have more rapid degradation rates, but this did not appear to be true. The caseins had little low molecular weight N material and rapid degradation rates, whereas the reverse was true for pot ale syrup and spent wash (Table 1). The difference in degradation rate between precipitated, freeze-dried casein and commercial casein is interesting. Both caseins are completely water-soluble, but preparation of the former may cause some denaturation, and thereby yield a more rapidly degraded product.

Degradation was not solely a function of protein solubility (Table 1). Degradation rates for soluble proteins ranged from values similar to those of oilseed meals (about .1 to .15/h) to rates as high as .8/h, relfecting the lack of homogeneity of degradation among soluble proteins, which also was reported by Mahadevan et al. (1980). Very slow degradation rates have been observed for the soluble proteins ovalbumin (Mangan, 1972; Wallace et al., 1987), bovine serum albumin (Nugent et al., 1983; Wallace, 1983; Broderick, 1987), bovine submaxillary protein(Nugent et al., 1983) and γ-globulins (Wallace, 1983). These slow rates may be attributed to the presence of intramolecular cross-linking in these proteins (Mahadevan et al., 1980; Wallace, 1983).

The degradation characteristics of nine protein meals (which are largely insoluble) were compared using the IIV and in situ methods (Table 2). Degradation rates ranged from .042 to .244/h and .014 to .067/h by IIV and in situ procedures, respectively. Significant degradation regressions were not obtained for feather meal and blood meal by the IIV method or for blood meal by the in situ procedure. Although rates obtained using the in situ method were much slower than by IIV (average 36%), the same ranking of these proteins was obtained by both methods. Degradation rates obtained by the two methods were highly correlated (r^2 = .919, Sy·x = .079, n = 7). An anomaly was observed with sunflower meal:its IIV rate (.058/h) was similar to that of copra meal and meat and bone meal, but its in situ rate (.032/h) was nearly twice that obtained for the other two meals (.017/h).

Rates determined by in situ methods may be expected to be lower than actual in vivo rates for at least three reasons: 1) microbial growth within in situ bags tends to reduce apparent rate of N loss (Nocek and Grant, 1987); 2) the microbial population inside the bag is restricted to numbers fewer than that of the surrounding digesta (Meyer and Mackie, 1986); and 3) rapid efflux from the bags of soluble, degradable protein leaves behind a residual protein that is more slowly degraded than the protein as a whole. Moreover, rapid efflux of protein not yet degraded may lead to overestimation of the degraded fraction (fraction 'a'). The validity of the in situ method depends on the assumption that net protein loss from the bags occurs only due to degradation.

These difficulties do not occur with the IIV system. The degradation rate is derived from the irreversible appearance of the degradation products, NH_3 and amino acids (from both soluble and insoluble proteins) as a consequence of degradation by the total microbial population over a 4-h period. Reduction of degradation due to end-product buildup is not a problem up to 4 h even for rapidly degraded proteins (Broderick, 1978).

Some potential problems of the IIV method, such as influence of fermentation inhibitors, were discussed previously (Broderick, 1987). It is recognized that IIV results are based on a relatively short incubation time, whereas in situ results are based on longer-term incubations. With proteins containing both rapidly and slowly degraded proteins, the rapidly degraded fraction may have a disproportionate effect on IIV degradation rates determined over this relatively short incubation period. The in situ method has the added advantage of reflecting

TABLE 2. PROTEIN DEGRADATION PARAMETERS OBTAINED WITH INHIBITOR IN VITRO (IIV) AND IN SITU INCUBATIONS

Protein	Degradation rate[a]		Intercept[b]		IIV degradability at ruminal passage, h^{-1} c			In situ degradability at ruminal passage, h^{-1} d		
	IIV (k_d)	In situ (c)	IIV (B)	In situ (b)	.02	.05	.08	.02	.05	.08
	h^{-1}		%							
Fish meal (ruminant)	.042	.014	81.9	68.6	74	55	46	60	46	42
Soybean meal	.166	.067	92.7	85.6	90	79	70	80	63	53
Linseed meal	.244	.077	94.7	79.8	93	84	77	84	69	59
Sunflower meal	.058	.032	87.7	81.0	78	59	49	69	51	42
Rapeseed meal	.124	.036	85.9	82.3	88	75	66	71	52	43
Copra meal	.050	.017	92.2	76.0	74	54	43	59	43	37
Meat and bone meal	.056	.017	88.2	60.6	77	58	48	67	55	50
Feather meal[e]	NS[f]	.004		77.6				35	28	26
Blood meal[e]	NS	NS								

[a]Fractional protein degradation rates determined by IIV (k_d) and in situ (c) methodology.

[b]Antilog of intercept from regression on time of log of fraction remaining undegraded.

[c]Extent of protein degradation estimated from IIV data (Broderick, 1987) at three assumed ruminal passage rates (k_p) using the equation: protein degradability, % = $(100 - B) + B\ [k_d/(k_d + k_p)]$.

[d]Extent of protein degradation estimated from in situ data (Ørskov and McDonald, 1979) at three assumed ruminal passage rates (k) using the equation: protein degradability, % = $(100 - b) + b[c/(c + k)]$.

[e]Feather meal did not yield a significant IIV degradation regression, so degradation parameters could not be computed. Blood meal did not yield significant degradation regressions with either the IIV or in situ methods.

[f]NS = Not significant.

ruminal conditions (such as pH) that influence protein degradation. However, IIV incubations can be altered to study the influence of such factors.

In situ and IIV rates observed in these studies may be compared to those reported by others for the same proteins. Degradation rates of .10 to .145/h were obtained with the IIV system for solvent-extracted soybean meal (Broderick, 1986, 1987). Erdman et al. (1987) reported an in situ degradation rate for soybean meal of .078/h; Miller (1982) found mean in situ rates of .102, .082 and .061/h for soybean meal, sunflower meal and "well-preserved" fish meal, respectively.

Estimated extents of degradation (Table 2), computed from in situ and IIV data, were more similar than degradation rates. Estimated extents of degradation (assuming ruminal passage of .05/h) were highly correlated to each other (r^2 = .754, Sy·x = 4.99, n = 7). Overall, mean in situ degradations for the seven procedures were 83.4% of those estimated from IIV data; only the in situ values for rapeseed meal diverged, averaging 72% of IIV data. Improved correspondence of results is due to the greater influence of the degraded fraction (the proportion of total N rapidly leaving in situ bags) in computation of in situ degradations. The degraded fraction 'a', estimated from the zero-time in situ intercept (a = 1 − b, where b is the intercept), averaged 23.7%; the corresponding fraction from the IIV assay (the proportion of total protein in the form of NH_3 and free amino acids at zero-time) averaged 11.0%. As a result, the intercepts and degradation rates determined in each system compensated for each other such that degradation estimates converged.

Based on in situ data and assumed ruminal passage of .05/h, ARC (1984) reported estimates of protein degradation of 22, 63, 59, 66, 78 and 45% for well-preserved fish meal, soybean meal, linseed meal, sunflower meal, rapeseed meal and meat and bone meal. The NRC (1985) published similar estimates of ruminal degradation, based on in vivo data, of 20, 72, 56, 76, 77 and 40%, respectively. Degradation was not given for copra meal protein in either publication. There probably will be some variation in degradation among commercial samples of protein sources. Nevertheless, extents of degradation found in our studies were of similar magnitude for five of these protein sources. However, our values for fish meal were more than twice those reported by the ARC (1984) and NRC (1985). Fish meal, which is "stale at processing," is reported to have a degradation of 52% (Mehrez et al., 1980), similar to the overall mean of IIV and in situ estimates of 54% from this study. It is difficult to assess technique reliability from so few data. However, our results were of similar magnitude to published extents of protein degradation, suggesting that both the IIV and in situ methods were satisfactory. Although in situ methodology is much simpler in application, the IIV procedure has the added advantage of yielding data for completely soluble proteins.

Using the in situ method, a very slow apparent rate of degradation was observed for feather meal; the slope from in situ N disappearance for blood meal was not negative; hence, a degradation rate could not be computed. Neither feather meal nor blood meal yielded significant negative regressions with the IIV procedure. Estimating gentle slopes (e.g. rates of less than .01/h) is statistically imprecise with either procedure. Moreover, the IIV method involves computing degradation from net (i.e., blank-corrected) release of NH_3 plus amino acids. More slowly degraded proteins give rise to smaller net release of breakdown products, and hence, estimates of slow degradation rates are less precise.

Conclusions

Ruminal protein degradation studies conducted with largely insoluble proteins indicated that rates of degradation determined by the in situ method averaged only 36% of those found using the IIV procedure. The IIV degradation rates were more consistent with reported in vivo rates of ruminal passage and extents of degradation. However, because in situ estimates of fractions degraded at zero-time were about twice as large as with the IIV method, extents of degradation computed from in situ data averaged 83% of IIV values. Both procedures yielded estimates of degradation that were similar to literature values for five of six protein meals. The IIV method may be used with soluble and insoluble proteins; neither procedure appears reliable for very slowly degraded proteins.

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